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SUPPLEMENTUM 20

On renal handling of plasma proteins

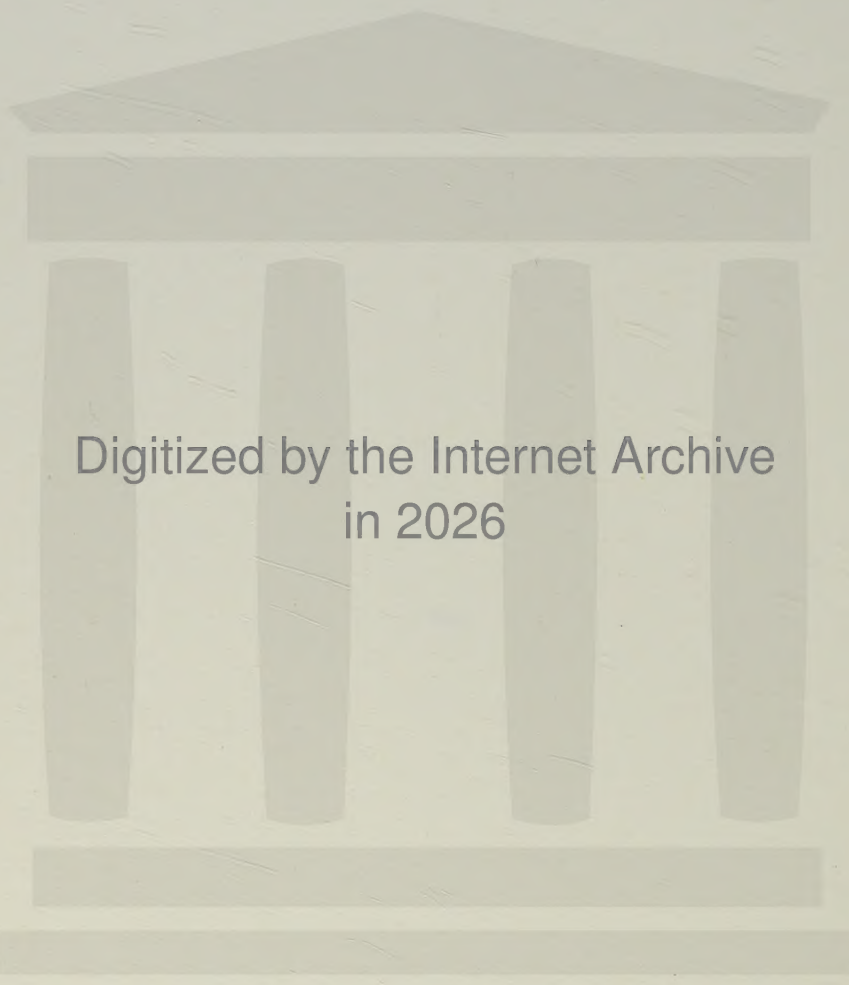
with special reference to α_2 -microglobulin,
 β_2 -microglobulin, lysozyme and albumin

By

UFFE RAVNSKOV

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AKADEMISK AVHANDLING

SOM MED VEDERBÖRLIGT TILLSTÅND AV
MEDICINSKA FAKULTETEN I LUND
FÖR AVLÄGGANDE AV MEDICINE DOKTORSEXAMEN
KOMMER ATT OFFENTLIGEN FÖRSVARAS PÅ FÖRELÄSNINGSSAL 3,
CENTRALBLOCKET, LASARETTET I LUND,
TORSDAGEN DEN 17 MAJ 1973 KL. 9.15

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UFFE RAVNSKOV

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SCANDINAVIAN JOURNAL OF
UROLOGY AND NEPHROLOGY
SUPPLEMENTUM 20

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On renal handling of plasma proteins

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 β_2 -microglobulin, lysozyme and albumin

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CONTENTS

Chapter I	INTRODUCTION	5
Chapter II	EARLIER STUDIES	6
	Glomerular permeability to protein	6
	Tubular reabsorption of protein	6
	Renal catabolism of protein	7
	Transtubular passage of protein	7
	Peritubular uptake of protein	8
	Tubular secretion of protein	8
	Causes to proteinuria in renal disease	8
Chapter III	PRESENT INVESTIGATION	10
Chapter IV	THE PROTEINS STUDIED	11
	Albumin	11
	α_2 -Microglobulin (retinol-binding protein)	11
	β_2 -Microglobulin	11
	Lysozyme	11
Chapter V	MATERIAL AND METHODS	12
	Clinical material	12
	Sampling and assays	12
	Proteins	12
	Antisera	13
	Immunochemical quantitation	14
	Other methods	14
	Calculations	14
Chapter VI	RESULTS AND DISCUSSION	15
	The methods used	15
	The serum level of LMW proteins (I, III)	16
	The protein/creatinine clearance ratios and GFR (I, II, III)	17
	Proteinuria after renal transplantation (III, IV)	18
	An alternative way of renal protein elimination (V)	20
Chapter VII	SUMMARY	22
	ACKNOWLEDGEMENTS	23
	REFERENCES	25
	APPENDIX	29

The present survey is based mainly on the results published or to be published in the following papers:

- I. B.G. Johansson & U. Ravnskov. The serum level and urinary excretion of α_2 -microglobulin, β_2 -microglobulin and lysozyme in renal disease. Scand J Urol Nephrol 6, 249, 1972.
- II. U. Ravnskov. Albuminuria in acute and chronic renal failure. Acta Med Scand, in press.
- III. U. Ravnskov. Proteinuria after human renal transplantation. 1. Urinary excretion of α_2 -microglobulin, lysozyme and albumin. Scand J Urol Nephrol, in press.
- IV. U. Ravnskov. Proteinuria after human renal transplantation. 2. A functional identification of two types of rejection crisis. Scand J Urol Nephrol, in press.
- V. U. Ravnskov, B.G. Johansson & J. Göthlin. Renal extraction of β_2 -microglobulin. Scand J Clin Lab Invest 30, 71, 1972.

LIST OF ABBREVIATIONS

- LMW: Low molecular weight.
- RBP: Retinol-binding protein (α_2 -microglobulin).
- GFR: Glomerular filtration rate.
- C_{Cr}: Creatinine clearance, ml/min/1.73m².
- FF: Filtration fraction; in paper V measured as E_{in}.
- E _{β} , E_{in}, E_{PAH}: The renal extraction rate of β_2 -microglobulin, inulin and p-aminohippurate, calculated according to the formula on p.
- C_{alb}/C_{Cr}, C _{α} /C_{Cr}, C _{β} /C_{Cr}, C_{lys}/C_{Cr}: The ratios between the clearance of albumin, α_2 -microglobulin, β_2 -microglobulin and lysozyme, and the clearance of creatinine. The formula used for the calculation is given on p.
- C_p/C_{Cr}: is used to denote the protein/creatinine clearance ratios for all four proteins, and
- C_{LMW}/C_{Cr} to denote only the ratios for the LMW proteins.
- FSE: Fractional sodium excretion, or sodium excretion in percentage of the filtered load. The formula for this calculation is given on p.
- FPE: Fractional potassium excretion.

I. INTRODUCTION

Proteinuria is one of the earliest and most common symptoms of renal disease. Knowledge of the way proteins are handled by the kidneys is therefore desirable. Such knowledge may be useful in the investigation of the pathogenesis and mechanisms of renal disease as well as in the appraisal of spontaneous or induced changes in renal function.

Despite praiseworthy advances in the last century the causes and mechanisms of proteinuria are not yet properly understood. This study concerns mainly the effect of the glomerular filtration rate (GFR) on protein excretion and evi-

dence of a new theory concerning the renal handling of low molecular weight (LMW) proteins.

The presentation and discussion of the papers forming the basis of this thesis is preceded by a short description of our present consensus of opinion of proteinuria. Literature on proteinuria is enormous and several recent reviews are available (Rather, 1952; Schultze & Heremans, 1966; Jensen, 1969; Rennie, 1970). Therefore, suffice it here to give the highlights of proteinuria in renal disease, and then mainly those papers bearing directly on the present study.

II. EARLIER STUDIES

The theoretical pathways by which plasma proteins enter and leave the kidney are shown schematically in fig. 1. In the following review these possibilities are considered essentially in the order shown in the figure.

GLOMERULAR PERMEABILITY TO PROTEIN

It is widely assumed, that in renal disease most urinary proteins enter the urine via the glomerular capillary membranes. This assumption has been corroborated by ultrastructural studies in experimental aminonucleoside nephrosis in rats injected with silver labelled bovine albumin

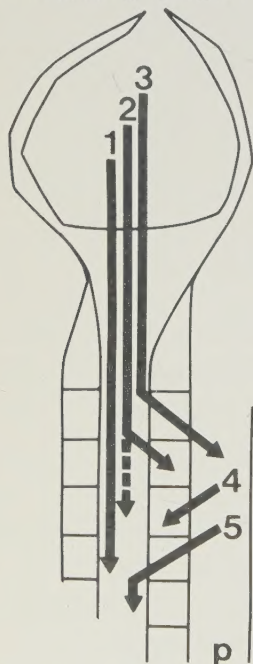


Fig. 1. The theoretical pathways of plasma proteins entering and leaving the kidney. 1. Glomerular filtration. 2. Glomerular filtration + tubular reabsorption and catabolism. The dashed arrow denotes incomplete tubular reabsorption (proximal tubular damage or load in excess of maximal reabsorptive capacity). 3. Glomerular filtration + transtubular passage of intact protein. 4. Peritubular uptake and catabolism. 5. Tubular secretion. p = peritubular vessels.

(Vernier, Papermaster, Olness, Binet & Good, 1960), by ferritin (Farquhar & Palade, 1961), horse radish peroxidase (Venkatachalam, Karnovsky & Cotran, 1969) and beef liver catalase (Venkatachalam, Cotran & Karnovsky, 1970).

Most authors agree that the passage of proteins through the glomerular membranes varies with the size and structure of the protein (Hardwicke, Cameron, Harrison, Hulme & Soothill, 1970), but neither the upper limit for total retention nor that for free filtration can be estimated without direct, reliable measurements of different protein concentrations in the capsular space, and as yet no such methods are available (see later).

Whether glomerular filtration of proteins can be explained by the pore theory of Pappenheimer (1953), or the diffusion theory of Chinard (1951), and whether the restricting barrier is the lamina densa of the glomerular basement membrane (Farquhar & Palade, 1961) or the pore slits (Graham & Karnovsky, 1966a), it is evident that if a transglomerular passage holds also for homologous proteins in human renal disease, the amount of protein which escapes through the glomeruli must apparently depend on the plasma concentration of the protein. Such a relationship, indirectly supporting the idea of a transglomerular transport of protein, has been found for hemoglobin (Monke & Yuile, 1940), myoglobin (Yuile & Clark, 1941), albumin (Chinard, Lauson, Eder, Greif & Hiller, 1954, Gregoire, Malmendier & Lambert, 1958) and lysozyme (Harrison & Barnes, 1970).

TUBULAR REABSORPTION OF PROTEIN

Normal urine contains only traces of protein (Berggård 1970). Therefore, those protein species which are believed to pass through the glomerular filter in notably larger amounts are

evidently lost on their way through the tubular system. Evidence of a proximal tubular reabsorption of protein has been produced (Rather, 1952), mainly in combined experimental and morphologic studies of pinocytosis and "droplets" of the proximal tubular cells. Morphologic identification of specific proteins has confirmed these earlier findings (Ericsson, 1964; Graham & Karnovsky, 1966 b; Maunsbach, 1966; Oliver & Essner, 1972). Also stop-flow experiments on dogs have pointed to the proximal tubular cells, but a slight reabsorption more distally has also been found (Aukland, 1960; Lathem, Davis, Zweig & Dew, 1960; Malmendier, DeKoster, Veiken, Brauman, Myttenaere & Lambert, 1960).

The occurrence of a tubular reabsorption of protein suggests that the abovementioned relationship between the plasma level and the urinary excretion is only valid above a certain plasma level, the threshold level, above which the tubular reabsorptive capacity is saturated. Such a threshold value has been demonstrated only for myoglobin (Yuile & Clark, 1941) and heterologous lysozyme in dogs (Harrison & Barnes, 1970) but this might, perhaps, be explained by the assumption that this threshold value is just above the normal plasma level and therefore demonstrable only for proteins not normally occurring in the plasma.

The assumption of a glomerular filtration of LMW proteins followed by proximal tubular reabsorption is strengthened by the observation of proteinuria in proximal tubular dysfunction caused by chronic cadmium poisoning (Friberg, 1950) or intrinsic renal disorders (Butler & Flynn, 1958), and the finding that the molecular weight of most of these proteins is below 30–50,000 (Friberg, 1950; Creeth, Kekwick, Flynn, Harris & Robson, 1963), the supposed maximal size of molecules capable of passing through the glomerular barrier (Hardwicke et al., 1970).

RENAL CATABOLISM OF PROTEIN

Most authors believe that proteins that are reabsorbed by the proximal tubular cells under-

go degradation, resulting in a higher level of amino acids and polypeptides in the blood from the renal vein than in blood from the aorta (Eliasch, Sellers, Rosenfeld & Marmorston, 1955; Sellers, 1956). No renal arteriovenous difference in total protein has been demonstrated (Cora, Debiassi, Rosa, Maggia & Tonello, 1962). Owing to the high renal plasma flow rate this fact does not exclude the possibility of extraction of substantial amounts of protein, provided the plasma concentration of the protein in question is reasonably high. A considerable arteriovenous difference of a protein present in only traces may, of course, escape attention when only concentrations of the total protein are measured.

The role played by the kidneys in the catabolism of proteins with a molecular weight above 50,000 seems to be limited. Thus, nephrectomy has only a minor effect on the turnover of albumin (Katz, Rosenfeld & Seller, 1960) and IgG (Wochner, Strober & Waldman, 1967). A definite prolongation of the disappearance rate of IgG has, however, been found in the rat (Truax & McCoy, 1965).

That the kidneys play an important role in the elimination of protein with a molecular weight below 50,000 is unquestionable, however. The survival of I^{131} -labelled Bence-Jones proteins (Solomon, Waldman, Fahey & McFarlane, 1965; Miettinen & Kekki, 1967; Waldman, Strober & Mogielnicki, 1972) and insulin (O'Brien & Sharpe, 1967) has accordingly been found to be greatly enhanced in patients with renal failure. Furthermore, the life time of the parathyroid hormone (Martin, Melick & Luise, 1969), Bence-Jones protein and L-chains (Wochner et al., 1967) and β_2 -microglobulin (Bernier & Conrad, 1969) is considerably prolonged in nephrectomised laboratory animals.

TRANSTUBULAR PASSAGE OF PROTEIN

Whether any *intact* protein is transported from the tubular lumen through the tubular cells is still debatable. The occurrence of a transtubular

passage of intact heterologous lysozyme in the flounder has recently been postulated (Maack & Kinter, 1969; Maack, Mackenzie & Kinter, 1971). But turnover studies of autologous lysozyme in the rat indicate that such transport, if any, is probably of only minor importance (Hansen, Karle & Andersen, 1971).

PERITUBULAR UPTAKE OF PROTEIN

Evidence of a renal uptake of protein besides the amount filtered has been produced by Zaharko, Beck & Blankenbaker (1966) and Chamberlain & Stimmler (1967). Both groups have found a renal extraction of insulin significantly exceeding the filtration fraction. Furthermore, the extraction rate was found to vary with the plasma level of insulin, indicating a renal regulation of the elimination of insulin.

Also other observations suggest the existence of a direct, renal uptake of protein. In the abovementioned studies of the elimination of different LMW proteins no significant difference in the survival rate of Bence Jones protein or L-chains (Wochner et al. 1967; Mogielnicki, Waldman & Strober, 1971) or β_2 -microglobulin (Bernier & Conrad, 1969) was observed between normal animals and animals in which the ureters had been ligated or severed. Although considerable filtration may persist after such procedures (Salomon & Lanza, 1962) a direct, renal uptake of these proteins cannot be excluded, as suggested also by Bernier & Conrad (1969).

The site of this renal, extraglomerular uptake of LMW protein is not known. Morphologic evidence of a passage of protein *via* the tubular basement membrane has been demonstrated by Oliver & Essner (1972), who used mushroom tyrosinase (mol.wt. 34,500) as an ultrastructural tracer in a study of glomerular permeability in the mouse. They occasionally found this substance within the basal infoldings of the proximal tubular cells, but did not draw any conclusions about its functional significance. The structure of the proximal tubules with their broad contact with a numerous of peritubular

capillaries (Griffith, Bulger & Trump, 1967) shows the existence of conditions for a peritubular uptake, *i.e.*, a transport of protein from the peritubular capillaries across the tubular basement membrane into the proximal tubular cells.

TUBULAR SECRETION OF PROTEIN

The "droplets" occurring in the cytoplasm of the proximal tubules were considered as a sign of tubular secretion of protein by some older pathologists (Rather, 1952). No convincing evidence for such a mechanism has been produced in recent years. Shuster, Jones & Flynn (1963) injected fluorescent human plasma protein and Bence-Jones protein into rats with one ureter ligated and found an equal quantity of fluorescence in the proximal tubular cells of controls and ureterligated kidneys and claimed that these proteins were secreted by the mammalian kidney. But this finding only demonstrates the presence of an uptake of these proteins into the tubular cells and is no proof of a tubular secretion.

CAUSES TO PROTEINURIA IN RENAL DISEASE

It would appear that a renal leakage of proteins of a molecular weight above 50,000 can be ascribed to an increased glomerular permeability to protein, most often due to immunological damage of the basement membrane. This pattern of proteinuria, dominated by medium size and large protein species is often called *glomerular proteinuria*. In contrast, an increased excretion of proteins of a lower molecular weight can probably be explained as an insufficient tubular reabsorption of these proteins, either because of damage to the proximal tubules or because of a tubular load exceeding maximal tubular reabsorptive capacity. This type of proteinuria has been designated *tubular proteinuria* (Butler & Flynn, 1958). For proteins in the intermediate molecular weight range both factors in combination may, of course, be operating.

These terms, glomerular proteinuria and tubular proteinuria, are used according to the electrophoretic protein pattern of the urine, whether glomerular or tubular damage has been proved or not. This fact should be kept in mind, as many factors are known which might induce or influence proteinuria in renal disease (Bing, 1935). Proteinuria has been observed after administration of epinephrine or other drugs causing renal vasoconstriction (Starr, 1926), renin (Pickering & Prinzmetal, 1940) and angiotensin II (Deodhar, Cuppage & Gableman, 1964) as well as steroids (Heyman & Grupe, 1969). A high-protein diet increases the output of protein in humans (Berglund, Scriver & Medes, 1935; Bing, 1935) as well as in rats (Rumsfeld, 1956), an effect which seems to be independent of the effect of urea on proteinuria (Rumsfeld, 1956; Finlayson & Bauman, 1956). Also fever, as well as acidosis may induce proteinuria (Berglund et al., 1935). The effect of these factors on proteinuria in renal disease is not properly understood.

The influence of GFR on proteinuria in renal disease has likewise received but little attention in spite of the fact that serious renal disease always implies a reduction of the GFR. Platt, Roscoe & Smith (1952) in a study of renal function after removal of renal tissue in the rat found proteinuria to be increased, especially when the reduced amount of nephrons was taken into consideration. An increased excretion of lysozyme (Wauters & Favre, 1970) and other LMW proteins (Harrison & Blainey, 1967; Harrison, Lunt, Scott & Blainey, 1968) was observed in patients with severe renal insufficiency. Also proteins of high molecular weight seem to be excreted in larger amounts in uremia. Thus Maiorca, Scarpioni, Cambi, Carrara & Dall'aglio (1967) found a more marked excretion of albumin, transferrin, IgG, α_2 -macroglobulin and IgM in uremic patients with pyelonephritis and glomerulonephritis than in such patients without uremia.

III. PRESENT INVESTIGATION

The concept of LMW proteinuria as a sign of proximal tubular disorder would be useful because the conventional clinical tests for proximal tubular function are laborious. But, then it would be necessary to distinguish between the effect of reduction, if any, of functioning nephrons and the renal disease *per se*.

Therefore, in the first paper (I) the excretion of the dominating proteins in LMW proteinuria, α_2 -microglobulin (retinol-binding protein), β_2 -microglobulin and lysozyme was determined and analysed in terms of clearance and related to the GFR in various renal diseases.

The inverse relationship found between the excretion of LMW proteins and the GFR (I) raised the question whether such a relationship also held for proteins of higher molecular weight. To exclude the influence of an increased glomerular permeability, this problem was studied mainly by analysis of urinary albumin excretion in non-glomerular renal diseases (II).

The urinary clearance of proteins has hitherto been studied mainly statically as single observations in different patients. In the analysis of the effect of the GFR on protein clearance a longitudinal study of proteinuria was made in patients

with substantial, rapid changes in the GFR. Patients undergoing a renal transplantation seemed to be the ideal material for such a purpose. At the same time it should be possible to assess the value of protein analysis in predicting rejection crisis. The excretion of the same four proteins as those studied earlier were therefore analysed sequentially in ten patients who had received a renal graft (III).

Patterns of proteinuria recorded in this investigation (III) made it possible to distinguish between two functional types of rejection crises. These findings were analysed (IV).

The relationship found between the serum level of β_2 -microglobulin and the GFR (I) proved incompatible with the earlier assumption that the serum level of LMW proteins depends solely on the GFR. It was thought that the discrepancy might be explained by a peritubular uptake of β_2 -microglobulin in addition to glomerular filtration followed by tubular reabsorption and catabolism. This possibility was tested by measuring the simultaneous renal extraction of β_2 -microglobulin, p-aminohippurate (PAH) and inulin in patients with different degrees of renal damage (V).

IV. THE PROTEINS STUDIED

ALBUMIN

Albumin was chosen because it is the preponderant urinary protein, it is easily detected even in normal urine with the methods used, and it occurs as a monomer in plasma as well as in urine, a prerequisite for clearance determinations. With a molecular weight of about 69,000 it is normally almost completely withheld by the glomerular filters and is therefore an adequate representative of the components in "glomerular" proteinuria.

α_2 -MICROGLOBULIN (retinol-binding protein, RBP)

This protein was chosen because it is one of the dominating proteins in tubular proteinuria. It has been isolated from normal plasma, where it circulates as a complex with prealbumin and retinol (Kanai, Raz & Goodman, 1968), and from urine in patients with proximal tubular disorders (Peterson & Berggård, 1971). In plasma it occurs both as a free protein and as the prealbumin-retinol-RBP complex. The ratio between these two forms was found to be markedly increased in patients with chronic renal disease (Smith & Goodman, 1971; Peterson 1971a), a finding which renders the value of RBP in clearance studies doubtful. However, even if absolute clearance levels may be unreliable, many of the characteristics of RBP in renal disease proved similar to those of the following two proteins and the results were therefore included in the present study.

β_2 -MICROGLOBULIN

This protein, isolated and characterized by Berggård & Bearn, has a molecular weight of about 12,000 and occurs in minute amounts and as a monomer in normal plasma and urine (Berggård & Bearn, 1968; Evrin, Peterson, Wide & Berggård, 1971). The elimination is considered mainly renal: glomerular filtration, followed by tubular reabsorption and catabolism, and in accordance herewith, a substantial rise in the plasma level has been seen in renal failure as well as a markedly increased excretion in proximal tubular disorders (Bernier, Cohen & Conrad, 1968; Peterson, Evrin & Berggård, 1969). Thanks to these properties it seems to be an ideal indicator of renal dysfunction.

LYSOZYME

The occurrence of lysozyme in urine has long been considered as a sign of tubular damage (Prockopp & Davidsson, 1964; Hayslett, Perillie & Finch, 1968; Wauters & Favre, 1970). The high lysozyme content of proximal tubular cells suggests that liberation of the enzyme from damaged tubular cells may also help to explain its occurrence in the urine in addition to those mechanisms causing an increased excretion of other LMW proteins. Comparison of lysozyme with its molecular weight of about 14,000 with other LMW proteins was therefore considered worth while and as it appeared largely as a monomer in plasma and urine from patients with renal failure (I) it was found suitable for clearance studies.

V. MATERIAL AND METHODS

CLINICAL MATERIAL

The present study was based on 140 patients with different renal diseases. Thirty-two of the patients were represented more than once. When being studied those patients undergoing renal transplantation were being cared for at the Department of Urology, the others, at the Renal Department of the University hospital of Lund.

The protein supply was not controlled, but no patient was on a protein-restricted diet. Except for the patients undergoing renal transplantation none received steroid treatment. A detailed description is given of the patients in the separate papers.

Twenty-five healthy individuals with no signs of renal disease served as controls.

SAMPLING AND ASSAYS

Urine samples from 24 h portions and blood samples obtained within this period, were analysed for their content of

α_2 -microglobulin, β_2 -microglobulin, lysozyme, albumin and creatinine. Urine from the transplanted patients was also analysed for total protein. Of the many analyses made at the routine laboratory of the hospital, only data from measurements of sodium and potassium in blood and urine are presented (IV). In the last investigation (V) inulin and p-aminohippurate were given intravenously and their levels were afterwards measured in arterial blood and in blood from the renal vein. The whole procedure is described in detail elsewhere (V).

PROTEINS

α_2 -Microglobulin was obtained from the urine of patients with severe renal failure. The protein was purified as follows: After concentration with polyethylene glycol of 1500 ml urine in Visking tubing 23/32 the urine proteins were separated by gel chromatography on Sephadex G 75 superfine (fig. 2). The fractions, which on agarose gel electrophoresis (Johansson, 1972) showed the typical pattern of

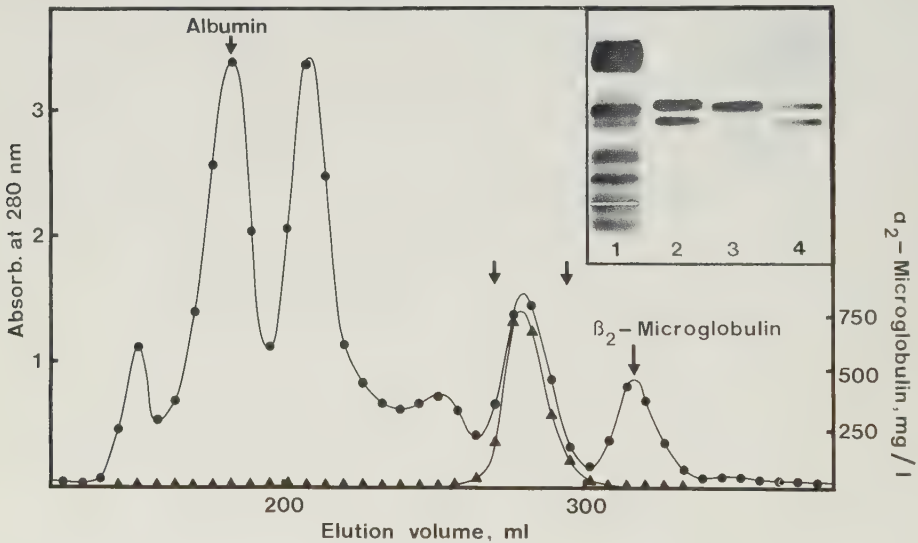


Fig. 2. Chromatography on Sephadex G 75 superfine of 4 ml concentrated urine from a patient with chronic pyelonephritis and severe renal failure. The column (90.5 x 2.5 cm) was equilibrated with 0.02 M Tris-HCl buffer, pH 7.3, containing 0.2 M NaCl. Fractions of 6.5 ml were collected at a flow rate of 13 ml/h. A total of 310 mg protein and 18 mg of α_2 -microglobulin was applied. The fractions between the arrows, containing a total of 16 mg α_2 -microglobulin, were pooled and concentrated for isoelectric

focusing. The maximal concentrations of albumin, α_2 -microglobulin and β_2 -microglobulin were determined immunochemically. Inset: Agarose gel electrophoresis of 1) the concentrated urine used for the α_2 -microglobulin preparation, 2) the pooled, concentrated fractions indicated by arrows on the graph (several faint bands became invisible on the photograph), 3) fraction 12, and 4) fraction 13 after isoelectric focusing (fig. 3).

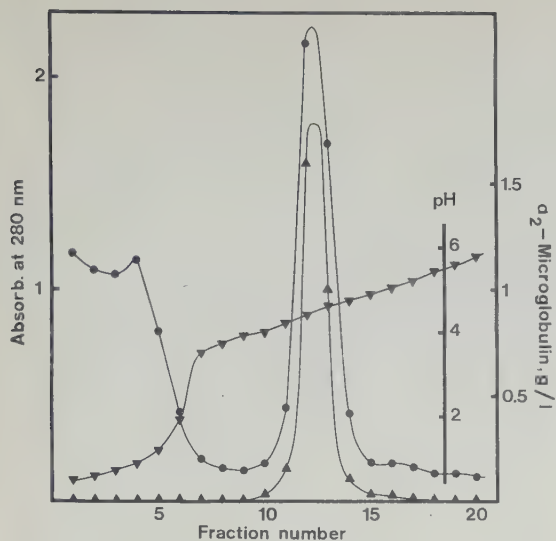


Fig. 3. Isoelectric focusing in an LKB 110 ml column containing Ampholine, pH 3–8 in a sucrose gradient. 2.4 ml of a fraction obtained by chromatography on Sephadex G 75 superfine (fig. 2), containing 9.7 mg of α_2 -microglobulin was applied. Fractions of 3 ml were collected after 32 hours focusing. Fraction 12 and 13 (fig. 2), containing a total of 7.2 mg α_2 -microglobulin were used as antigen.

α_2 -microglobulin (fig. 2), were concentrated and dialysed 48 hours against a 1% glycine solution, followed by 30 hours isoelectric focusing in an LKB 110 ml column according to the instructions given by the manufacturers. Ampholine, pH 3–8 in a sucrose gradient was used for the separation. Fractions of 3 ml were collected and the maximal α_2 -microglobulin concentration was found in the pH-range between 4.2 and 4.4 (fig. 3). On agarose gel electrophoresis the final α_2 -microglobulin preparations showed 2 major and 1 minor components, which proved to be immunochemically identical on crossed immunoelectrophoresis (Laurell, 1965; Ganrot, 1972). The latter procedure was also used for analysis of concentrated urine samples from several different patients. Two up to five immunologically identical components could be found. These components always appeared in the same fractions on gel chromatography and thereby indicated that the different components were of a similar molecular size.

The concentrations of the pure α_2 -microglobulin was calculated from its absorbance at 280 nm (Peterson & Berggård, 1971).

Lysozyme was prepared from urine of patients with monocyte leukemia (Johansson & Malmquist, 1971).

β_2 -Microglobulin was prepared from urine samples from patients with severe renal failure using the same procedure as that described by Berggård & Bearn (1968).

ANTISERA

Antisera against α_2 -microglobulin and lysozyme were prepared in rabbits by injecting about 1 mg of the antigen emulsified with complete Freund's adjuvant into the hind-foot pads. Booster injections were given after 3 and 6 weeks. No absorption was necessary as the antisera were apparently monospecific in crossed immunoelectrophoresis against normal and uremic sera, and against pooled, concentrated urine from uremic patients (fig. 4).

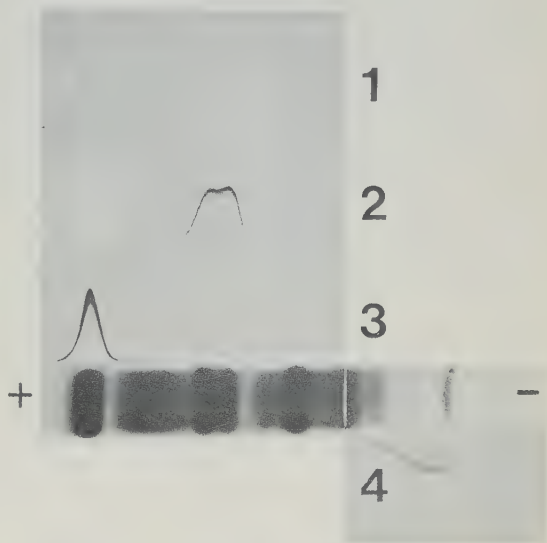


Fig. 4. Crossed immunoelectrophoresis of concentrated urine from a patient with severe uremia against the antisera used in the investigation: 1. Anti- β_2 -microglobulin, 2. Anti- α_2 -microglobulin, 3. Anti-albumin, 4. Anti-lysozyme. Top: Immunoelectrophoresis of the same urine against anti- β_2 -microglobulin. The concentrations of the antisera in the crossed immunoelectrophoresis were the same as used in the quantitative determinations, whereas the concentration of the urine was chosen to give precipitates of a convenient size.

The antisera against β_2 -microglobulin and albumin were kind gifts from Dr. G. Lindstedt and Professor C.-B. Laurell to Dr. B. G. Johansson. The antiserum against albumin was apparently monospecific in crossed immunoelectrophoresis, whereas the antiserum against β_2 -microglobulin showed a few faint, additional precipitates on immunoelectrophoresis (Scheidegger, 1955) against pooled concentrated urines, but not on crossed immunoelectrophoresis (fig. 4). The investigation was performed with this antiserum throughout. The antiserum was later absorbed with normal serum and a cathodal fraction from preparative electrophoresis of concentrated urine from a patient with uremia, and the β_2 -microglobulin levels in 20 different serum and 20 urine samples were determined both with the resulting

monospecific antiserum and the antiserum used in the investigation, but no significant difference between the values could be measured.

IMMUNOCHEMICAL QUANTITATION

Albumin and β_2 -microglobulin was determined with single radial immunodiffusion according to Mancini, Carbonara & Heremans (1965) with the following modifications: The plates were prepared by pouring 25 ml of a warm, antibody-containing solution of 1% agarose (Miles-Sevarac, Maidenhead, England) in 0.02 M Tris-HCl buffer, pH 7.3, containing 0.2 M NaCl, on a strictly horizontal 11 x 20 cm glass-plate. Circular wells were punched with a stainless steel tube with a 4 mm bore and in each of the wells 10 μ l of a sample or standard solution was deposited with the aid of a Hamilton syringe built into a device described by Laurell (1972). The plates were kept in a moist box at room temperature during the diffusion time (24 h for the β_2 -microglobulin, 48 h for the albumin assay). Sodium azide (0.05% w/v) was added to the agarose solution. The plates were washed for 24 h with saline and dried. Coomassie brilliant blue R-250 was used for staining β_2 -microglobulin, amido black for albumin. The diameters of the precipitation rings were measured in two perpendicular directions and the mean value was used as a measure of the antigen content of the solutions.

Lysozyme and α_2 -microglobulin was determined by use of electroimmunoassay (rocket immunoelectrophoresis) according to Laurell (1966; 1972).

As standards in the immunochemical methods were used concentrated urine from uremic patients. The content of the proteins of these standard solutions was determined by use of the pure proteins.

OTHER METHODS

Total protein was measured by precipitating the protein of a 2 ml urine sample with trichloroacetic acid followed by photometric quantitation after biuret reaction on the precipitate.

Creatinine was determined by means of an autoanalyzer technique (Charson, Grady & Stanley, 1961).

Inulin was determined by the method of Heyrovsky (1956), and PAH according to Brun (1951).

The normal levels, the precision and the detection limits of the assays are given elsewhere (I, II, III, V).

CALCULATIONS

Urinary protein excretion was calculated as the ratios between the clearance of the protein and clearance of creatinine, according to the formula:

$$\frac{U_P \cdot V/S_P}{U_{Cr} \cdot V/S_{Cr}}$$

where U_P and U_{Cr} denote the urinary concentration of protein and creatinine. S_P and S_{Cr} the serum concentrations and V urine volume (output per unit of time). As V cancels out the simplified equation becomes:

$$\frac{U_P/S_P}{U_{Cr}/S_{Cr}}$$

In paper IV the urinary sodium excretion was calculated in the same way:

$$\frac{U_{Na} \cdot V/S_{Na}}{U_{Cr} \cdot V/S_{Cr}} \quad \frac{U_{Na}/S_{Na}}{U_{Cr}/S_{Cr}}$$

also called fractional sodium excretion (FSE).

The renal extraction rate of a substance X was calculated according to the formula: $E_X = (A_X - V_X):A_X$, where A_X and V_X are the concentrations in arterial and renal venous blood.

VI. RESULTS AND DISCUSSION

THE METHODS USED

The mechanisms by which the kidneys regulate the homeostasis of the body have been elucidated mainly by analysis of tubular fluid obtained by micropuncture at different levels of the nephron. Several attempts to elucidate the renal handling of proteins in this way have been made since the first successful puncture of the glomerular capsule of the frog. However, the large difference in protein concentration between the tubular lumen and the surrounding extracellular fluid may give rise to substantial errors owing to contamination of the tubular fluid with extraneous plasma albumin (Oken & Flamenbaum, 1971). All reliable quantitative studies of renal handling of proteins have therefore hitherto been based on analysis of blood and urine.

When analysing such data the peculiar structure of the kidney must be considered. It is obvious, but often forgotten, that the urine output teaches nothing about the amount of nephrons participating in the production of urine. Without knowledge of the number of functioning nephrons, conclusions based on plasma and urine levels of protein may therefore be misleading. Berglund et al. (1935) and Bing (1936) were the first to realise that proteinuria depends both on the glomerular permeability and the area of the glomerular membranes, and therefore related the amount of protein excreted per day to the GFR.

Bing and many of his contemporaries thought that protein was filtered and neither secreted nor reabsorbed by the tubules. Though evidence of a tubular reabsorption of protein has since been produced, it need not invalidate their method of calculation: whatever happens to the protein in the nephron, it is essential to know the number of participating nephrons for the simple reason that the protein leakage of the

average nephron at a given protein excretion must vary inversely with the number of functioning nephrons.

As mentioned earlier, the amount of protein excreted in the urine depends also on the load of the protein presented to the nephron, *i.e.* to the plasma level of the protein. It is therefore necessary to include also this value in the clearance formula, given on p. 14.

The equation is independant of sampling errors, as volume cancels out volume, but to eliminate postural influence on protein excretion, the urine samples should be standardized, either by using urine produced during recumbency only, or an aliquot of the 24 hours urine output. The latter method, which was used in the present study may, of course, be influenced by incomplete collection of urine, if the protein content of a missing sample should deviate markedly from the 24 hours mean, but this theoretical possibility was ignored as also the simultaneous measurement of the clearance of creatinine was desired.

The value of this method of calculating was demonstrated by Chinard et al. (1954), who found that a parenteral supply of albumin to nephrotic patients with low plasma albumin levels raised both the plasma albumin level and the absolute amount of albumin excreted, but had no influence on the albumin/creatinine clearance ratio. Hardwicke et al. (1970) showed that the increase in albumin excretion with age in healthy children depended on a simultaneous increase of the GFR and not on the albumin/creatinine clearance ratio. The same group showed that this ratio eliminates the influence of a rising level of serum albumin or a rising GFR on albumin excretion in children with chronic glomerulonephritis.

These investigations thus show that the protein/creatinine clearance ratio is not affected by changes in protein excretion not caused by changes in the function of the nephron itself, but by variation in the load or in the number of nephrons.

The significance of the equation depends on the precision to which CC_R reflects the GFR. The possibility that CC_R overestimates the GFR does not affect the main conclusion of the present work, the inverse relationship between GFR and C_p/C_{Cr} , as an overestimation, if any, of GFR due to urinary excretion of creatinine in excess of the filtered load is compensated by a corresponding underestimation of the mean protein excretion per nephron, expressed by the ratio C_p/C_{Cr} .

The plasma concentration of a protein is obviously not equivalent to the load if only part of the protein is exposed to glomerular filtration, such as in polymerisation or complex formation to larger molecular species. As mentioned earlier, this requirement is not met by α_2 -microglobulin, as it has been claimed to associate with prealbumin. Theoretically, the same phenomenon may cause an underestimation of the serum value of α_2 -microglobulin when free RBP is used as standard. However, as the complex is found to dissociate in an electric field (Kanai et al., 1968) and in the presence of antibodies to RBP (Peterson, 1971), the techniques used probably measure the total content of RBP molecules also in serum.

The *precision* of the immunochemical protein determinations with a coefficient of variation between 4 and 8% seems sufficient, as protein excretion was found to increase almost exponentially with increasing renal failure (I, II). The *accuracy* of the protein determinations is of minor importance, as clearance measurements express ratios between the serum and the urine concentrations.

THE SERUM LEVEL OF THE LMW PROTEINS (I, III)

As mentioned earlier, the elimination of LMW proteins is retarded in renal failure, probably because the catabolism of these proteins is mainly renal. Accordingly, an inverse relationship was found between the serum level of the LMW proteins and CC_R .

Impairment of renal function should retard elimination of a small protein more than that of a large one owing to the smaller relative amount of the latter presented to the tubules. Therefore, as expected, a low CC_R had the strongest effect on the serum level of the smallest

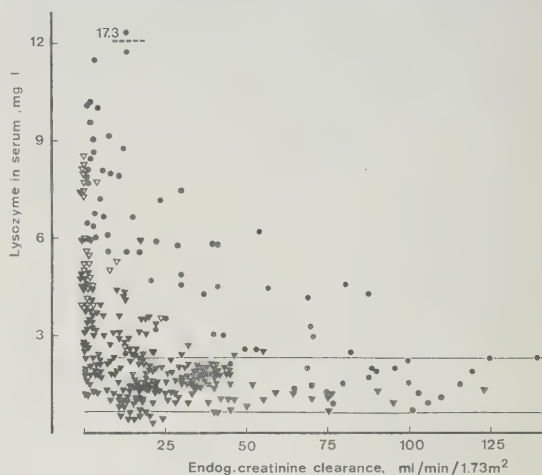


Fig. 5. Relation between the serum lysozyme concentration and CC_R in various renal diseases (●) and in patients with a renal graft during the first three days after the transplantation and the beginning of the immunosuppressive therapy (▽), and during the subsequent period (▼).

molecule studied, β_2 -microglobulin. In fact, in all patients with a reduced CC_R , the serum level of β_2 -microglobulin was raised, a finding in accordance with earlier observations (Bernier, Cohen & Conrad, 1968; Peterson et al., 1970). This phenomenon was not found for lysozyme despite only a minor difference in molecular weight between this protein and β_2 -microglobulin. This suggested that other factors, probably extra-renal, are responsible for the serum level of lysozyme in renal failure.

The relationship between CC_r and the serum level of RBP is obviously more complicated, as reflected in the wide inter-individual variations in the serum level in severe renal failure. A retention of free RBP as claimed by Kanai et al. (1971) and Peterson (1971 b) may be masked by unknown changes in the level of the whole prealbumin-RBP-retinol complex.

The serum level of the LMW proteins in the patients with a renal graft showed essentially the same dependence on CC_r , which, here too, was most evident for β_2 -microglobulin (fig. 1—3 in I). When the serum levels were plotted against CC_r , as in I, the lysozyme values were lower than those in the patients with different renal diseases except in the first two or three days after the transplantation (fig. 5). No explanation can be offered for this phenomenon, though immunosuppressive therapy may, perhaps, also influence the synthesis of lysozyme.

THE PROTEIN/CREATININE CLEARANCE RATIOS AND THE GLOMERULAR FILTRATION RATE (I, II, III)

Proteinuria is associated mainly with glomerular diseases, but a common clinical experience is that in renal failure also non-glomerular renal diseases are sooner or later accompanied by an increased excretion of proteins. Yet the intimate relationship between GFR, expressed as CC_r , and C_p/CC_r which was found in all proteins studied, was surprising. The reason why this phenomenon has hitherto received but little attention is probably the reduction of the number of nephrons, which may compensate for the increase of the mean protein excretion per nephron, when protein excretion is expressed in the conventional way in grams per 24 hours.

The correlation between CC_r and C_p/CC_r was weakest for β_2 -microglobulin and strongest for albumin. In view of the high serum levels of β_2 -microglobulin and the insignificant changes in serum albumin in severe renal failure it would appear that an increased tubular load of the

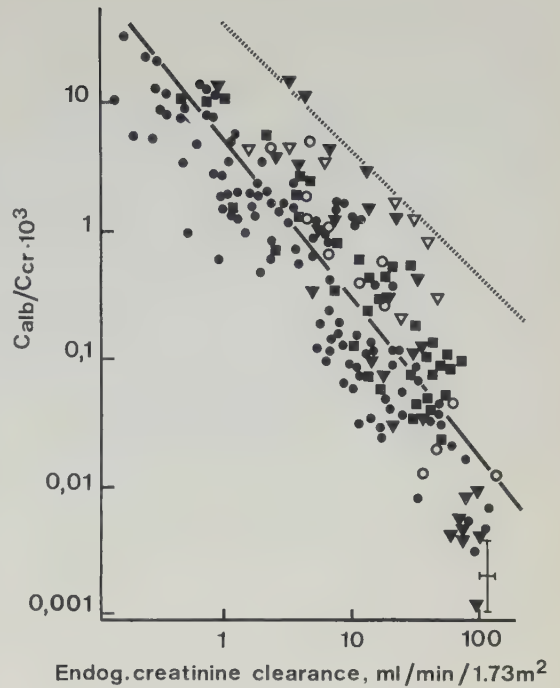


Fig. 6. Relation between CC_r and C_{alb}/C_{Cr} in non-glomerular renal disease. $\blacktriangledown$ Chronic pyelonephritis. $\circ$ Polycystic kidney disease. $\blacktriangledown$ Gouty nephritis. $\circ$ Acute renal failure. $\blacksquare$ Transplanted kidneys. — Regression line for all non-glomerular renal diseases, ----. Regression line for 31 patients (not shown here) with chronic glomerulonephritis. The cross in the lower, right hand corner denotes mean ± 2 SD in 25 healthy individuals.

proteins in excess of tubular reabsorptive capacity is not the main cause of this relationship.

The close relationships between the clearance of the LMW proteins were evident, even though considerable discrepancies were found in some patients (I). A positive correlation was found between the clearance of albumin and that of the LMW proteins only when patients with glomerulonephritis were excluded, and even then the scatter of the values was wide, indicating that different mechanisms are involved behind the increased excretion of the different proteins. Patients with gouty nephritis did not have a LMW protein excretion in parity with their excretion of albumin, whereas no specific patterns were noted in patients with other renal diseases.

The inverse relationship between C_{alb}/C_{Cr} and C_{Cr} varied from one renal disease to another as the slopes of the regression lines in a log-log graph for patients with pyelonephritis, polycystic kidney disease, acute renal failure, after a renal transplantation and with glomerulonephritis decreased in the order given. The differences between the slopes of the non-glomerular renal diseases were small, but significant between the groups with the largest and smallest gradients. They may possibly reflect different mechanisms of albuminuria between the various non-glomerular renal diseases.

In contrast, the excretion rate of LMW protein varied but little with the type of renal disease. An increased tubular load, owing to an increased tubular flow and/or a raised serum level of the proteins, in combination with uremic intoxication of the proximal tubular cells, factors common to all types of renal diseases, may be operating, but the number of patients in each disease group in this study was perhaps not large enough to exclude any differences between the groups.

A decreased renal elimination of LMW proteins in renal failure despite an increased urinary excretion appears paradoxical, but not if it be realised that filtered protein whether reabsorbed or not, is lost from the organism.

The absolute levels of the C_{alb}/C_{Cr} in the patients with glomerulonephritis were higher and the regression coefficient smaller than in the non-glomerular renal diseases, probably because the dominant cause of albumin excretion in glomerulonephritis is an increased glomerular permeability to macromolecules, irrespective of the degree of renal failure. It is interesting to note, however, that in many patients with renal failure caused by non-glomerular renal disease C_{alb}/C_{Cr} exceeded that of patients with glomerulonephritis (fig. 6).

In the patients with pyelonephritis a sufficient number of patients were studied to warrant a comparison between those with hypertension and those without, those with urinary tract in-

fection and those without, and finally those with a history of analgesic nephropathy and those without. No difference in the ratio between C_{alb}/C_{Cr} and C_{Cr} was seen between these groups indicating that these factors are of minor importance as causes of an increased albumin excretion.

The common view that an increased excretion of LMW proteins reflects a damage to the proximal tubules was not disproved in the present study. No attempt have been made in the past, however, to correlate the excretion of LMW proteins with the usual parameters of proximal tubular function. As long as other causes, such as those suggested earlier, of an increased LMW protein excretion have not been excluded, it seems necessary to be satisfied with the statement of an increased excretion of LMW proteins.

PROTEINURIA AFTER RENAL TRANSPLANTATION (III, IV)

The value of specific protein analysis and of the calculation of C_p/C_{Cr} was demonstrated in a study of proteinuria after renal transplantation in 10 patients.

The inverse relationship between C_{Cr} and C_p/C_{Cr} again resulted in a sharp decrease of C_p/C_{Cr} following the rapid postoperative improvement of renal function, and a similar abrupt increase of C_p/C_{Cr} simultaneously with the decrease of C_{Cr} in association with a rejection crisis. A distinct exception in the excretion pattern of the LMW proteins was, however, noted during 6 of 11 rejection crises observed. These 6 crises differed also in other respects. According to the LMW proteinuria pattern a functional distinction between two types of rejection crises, here referred to as type I and type II crises, could be made.

During the first 2–3 days of a type I crisis C_{LMW}/C_{Cr} decreased in spite of a substantial decrease in C_{Cr} , and in contrast with a simultaneous increase in C_{alb}/C_{Cr} . Not until day 3 or 4 did a marked increase in the LMW protein

excretion occur. Type I crises were characterized further by:

1. early onset (before day 12 in 5 of 6 crises),
2. a substantial reduction in urine output, and
3. a marked decrease in sodium output parallel to the decrease in LMW protein excretion (5 of 6 crises).

During a type II crisis C_p/C_{Cr} for all four proteins increased in accordance with the rule of an inverse relationship between C_{Cr} and C_p/C_{Cr} . Type II crises could be characterized further by:

1. late onset (all crises started later than day 13),
2. no significant reduction in urine output, and
3. no significant decrease in sodium output.

It was demonstrated that the decrease in sodium excretion during type I crises was not caused by the low urine output: also the fractional sodium excretion (FSE), *i.e.*, sodium excretion in percentage of the filtered load, was diminished significantly, especially evident when FSE was related to the GFR, whereas no significant change in FSE was seen during type II crises.

The mechanisms of these opposite changes of different protein clearances is not clear. An increased excretion of LMW protein is usually regarded as a sign of proximal tubular damage. The observed decrease of LMW protein excretion during rejection crisis, a situation of progressive renal damage, suggests the occurrence of other factors determining the excretion of the LMW proteins. This can possibly be explained by prolongation of the contact between the tubular fluid and the cells lining the tubular system, caused by a diminished renal blood flow (Hollenberg, Retik, Rosen, Murray & Merrill, 1968), thus resulting in an enhanced reabsorption of both LMW protein and sodium.

Whatever the correct explanation, such changes can evidently not be detected by measurement of the total protein excretion, which is

the conventional clinical measure of proteinuria (fig. 7).

Determination of lysozyme in urine has been considered of value in predicting a rejection crisis (Noble, Najarian & Brainerd, 1965; Han-

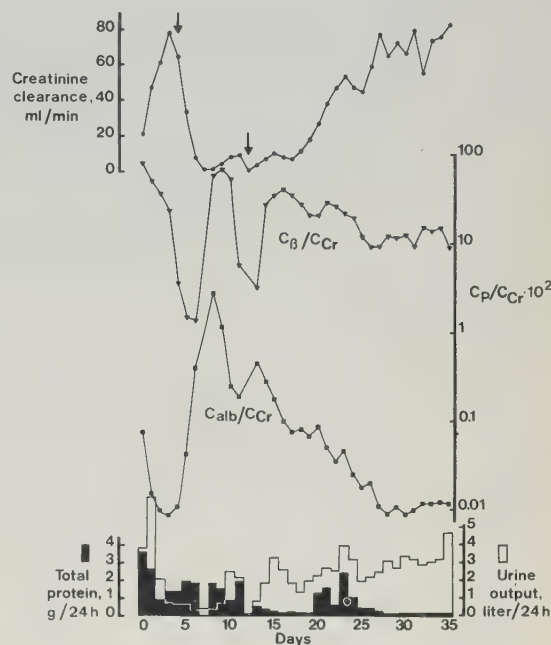


Fig. 7. The postoperative course of a patient (BA) who received a renal graft on day 0. Beginning of a type I crisis on day 4 and on day 12 (arrows). Total protein not measured on day 7 and day 12.

sen & Weeke, 1970; Shehadeh, Carpenter, Monterio & Merrill, 1970) but not by others (Harrison, Barnes & Blainey, 1972). As all available information on this subject is based on measurements of the urinary level of lysozyme without consideration of the influence of the actual GFR, objections may be raised against the value of these observations. In the present investigation (III) neither the lysozyme nor the excretion pattern of the other proteins were found to be of value for this purpose. Changes in the patterns occurred but always concomitantly with the reduction of C_{Cr} .

AN ALTERNATIVE WAY OF RENAL PROTEIN ELIMINATION (V)

The relationship between the serum level of β_2 -microglobulin and C_{Cr} found in paper I suggested that the elimination of this protein was not a simple question of glomerular filtration. According to the classical clearance formula, $C = UV/P$, the plasma level P of a substance, whose elimination depends only on glomerular

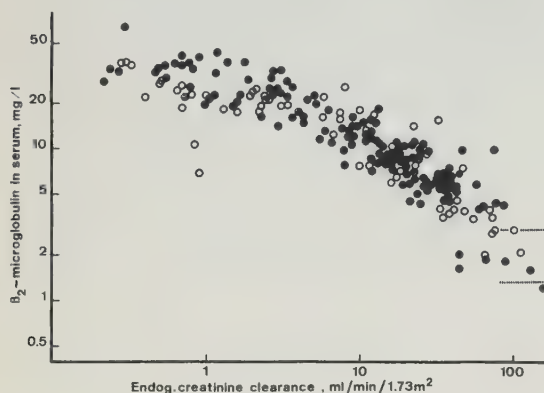


Fig. 8. Relation between serum level of β_2 -microglobulin and C_{Cr} in 6 patients with acute renal failure (●) and in 10 patients after renal transplantation (○). The dashed lines denote the mean serum level $\pm$ 2 SD in 25 healthy persons.

filtration, is a reciprocal exponential function of its clearance C , assuming a constant production of the substance in question. Consequently, the regression line calculated from the log values should be linear, as for creatinine, but the same regression line for β_2 -microglobulin was convex upwards, both when many solitary values from different patients (I) and when sequential values from a few patients with substantial changes of GFR were plotted (fig. 8).

Several explanations of this phenomenon may be offered, one of which is that it might be an effect of renal disease or renal failure on the production of β_2 -microglobulin, but a more likely one is the existence of a renal elimination of β_2 -microglobulin in addition to glomerular filtration, as evidence of the latter mechanism has been produced in studies of the renal handling of insulin, another LMW protein

(Zaharko et al., 1966; Chamberlain & Stimmler, 1967).

Such a mechanism would apparently imply that more β_2 -microglobulin is eliminated by the kidneys than can be explained by glomerular filtration alone, *i.e.*, that the extraction rate of β_2 -microglobulin (E_β) exceeds the filtration fraction (FF).

Preliminary studies of E_β gave values close to the normal FF. This did not exclude a minor extraction in excess of FF since the latter may vary widely, especially in renal disease, but demonstration of such extraction would require measurement of the actual FF simultaneously. The proximal tubules were thought to be the most probable site of a non-glomerular uptake of LMW proteins. Measurement and correlation of β_2 -microglobulin uptake with the extraction rate of p-aminohippurate (E_{PAH}) might thus also be of value.

Such measurements in patients with varying degrees of renal damage showed that E_β in kidneys with normal E_{PAH} often exceeded FF, measured as E_{in} . Furthermore, a decreased ratio between E_β and E_{in} was seen when E_{PAH} decreased. This ratio should evidently be about 1, and should be unaffected by proximal tubular function, or, more correctly, by the extent to which PAH has been eliminated from the blood, if β_2 -microglobulin is eliminated by glomerular filtration only.

These figures seem difficult to explain without the assumption of a renal uptake of β_2 -microglobulin in addition to, and independent of, glomerular filtration, probably located to the proximal tubules.

As E_β was only 40–50% of E_{in} in the patients with a decreased E_{PAH} , the glomerular filtration of β_2 -microglobulin seems to be considerably lower than commonly held. It also shows, that the amount of β_2 -microglobulin eliminated by the non-glomerular way in patients with a normal proximal tubular function is of a similar

magnitude as that eliminated by glomerular filtration and tubular reabsorption.

A peritubular uptake would allow a rapid, renal elimination of LMW proteins, some of which may serve important biologic functions. In addition, this mechanism may add a renal regulatory function to the elimination of LMW

proteins, which would not be possible if glomerular filtration were the only way of elimination. Corroboration or refutation of this assumption must abide further research, however. Likewise, the presumed peritubular localisation might be demonstrated by appropriate morphologic techniques, either with the use of electron-dense LMW proteins or fluorescent antibodies.

VII. SUMMARY

Observations made in the present investigation suggest that small but definite changes of separate renal functions may be discovered by studying the urinary excretion of protein, provided that this excretion is expressed as the protein/creatinine clearance ratio, calculated from immunochemical analysis of well-characterized protein species. With the use of specific antisera against α_2 -microglobulin (retinol-binding protein), β_2 -microglobulin, lysozyme and albumin, the value of this method was demonstrated in a study of proteinuria in the most common renal diseases and after renal transplantation.

An increased excretion of all four proteins was seen in all kinds of renal disease with a reduction of the glomerular filtration rate. Due to this phenomenon the urine from patients with severe renal failure was found a convenient source for the isolation of the low molecular weight (LMW) proteins.

An inverse relationship was found between the protein/creatinine clearance ratio of all proteins and the clearance of creatinine (C_{Cr}) in all conditions studied, both when values from different patients were plotted in a log-log graph and in sequential studies of patients with substantial impairment of renal function.

No difference in LMW protein excretion between various renal diseases could be noted, whereas the slope of the regression line between the albumin/creatinine clearance ratios (C_{alb}/C_{Cr}) and C_{Cr} differed significantly between different renal diseases.

Hypertension, urinary tract infection or a history of misuse of analgesics did not seem to influence albumin excretion in chronic pyelonephritis.

An increased albumin excretion during and after rejection crises after renal transplantation was detectable only by relating C_{alb}/C_{Cr} before and after the rejection crisis to C_{Cr} .

Opposite changes of the clearance of albumin and the three LMW proteins were recorded during early rejection crises, changes which permitted a distinction between two types of rejection crises. The protein excretion patterns were of no value in predicting the rejection crises.

A close relationship between the serum level of β_2 -microglobulin and C_{Cr} was found. The character of this relationship was incompatible with existing theories of renal handling of this protein. Determinations of the renal extraction rate of β_2 -microglobulin (E_β), p-aminohippurate (EPAH) and inulin (E_{in}) simultaneously in patients with different degrees of renal damage revealed that E_β exceeded the filtration fraction, measured as E_{in} , in kidneys with a normal EPAH. Furthermore, a positive correlation was found between E_β in proportion to E_{in} , and EPAH. The ratio between E_β and E_{in} indicated that the glomerular filtration of β_2 -microglobulin may be considerably below that of inulin.

The latter results suggest a renal uptake of β_2 -microglobulin in addition to, and independent of, glomerular filtration.

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APPENDIX

The present investigation includes no absolute levels of any substance in the urine. Therefore, a few primary data are given in this appendix for exemplification.

Table 1 shows data on the patients with chronic pyelonephritis; table 2, data on one of

the patients who had received a renal graft.

To calculate C_p/C_{Cr} , FSE and FPE, the serum values of the proteins, of sodium and of potassium were plotted against time, and the values used in the formulas were read from the middle of each 24 h sampling period.

Table 1. Pertinent data on 28 patients with chronic pyelonephritis, described in detail in paper II. Patients marked with an asterisk also included in paper I.

Patient	MA*	EA*	SA*	HB*	LB	EC*	EE*	ME	C
Sex	F	F	F	M	M	M	F	F	
Age	51	57	45	62	50	39	60	25	
Blood pressure	(+)	+		+	+	+	—	—	
Abuse of analgesics	yes	no	no	yes	no	yes	yes	no	
Urinary tract infection	yes	no	yes	yes	no	yes	yes	yes	
Creatinine, serum, mg/l	14	28	79	33	13	51	12	51	
Creatinine clearance, ml/min/1.73 m ²	44	25	5.8	22	95	15	40	14	
Albumin, serum, g/l	32	40	43	50	43	44	41	49	
Albumin, urine, mg/l	5	630	220	180	6	40	72	1290	
Albumin, urine, mg/24 h	6	1500	400	450	4	80	75	3000	
C _{alb} /C _{Cr} · 10 ³	0.004	1.26	1.08	0.3	0.001	0.1	0.034	3	0.0
α_2 -microglobulin, mg/l, serum	74	99	157	166	75	138	94	130	
α_2 -microglobulin, mg/l, urine	ND	13	64	24	ND	30	2.8	24	
C _{α} /C _{Cr} · 10 ²	—	1	8.7	1.2	—	2.3	0.16	2.4	
β_2 -microglobulin, mg/l, serum	3.8	17	13	7.6	2.9	8.8	5.9	7.8	
β_2 -microglobulin, mg/l, urine	ND	7	39	17	0.4	35	11	13	
C _{β} /C _{Cr} · 10 ²		3	63	18	0.12	42	3.5	24	
Lysozyme, mg/l, serum	3	3.5	8.1	3.2	—	6.7	5.8	—	
Lysozyme, mg/l, urine	ND	ND	4.8	0.6		2.4	0.5	—	
C _{lys} /C _{Cr} · 10 ²	—		12	1.2		3.8	0.17	—	

Table 2. Pertinent data from a patient (BA) who received a renal graft on day 0. See also fig. 7.

Day	0	1	2	3	4	5	6	7	8	9
Creatinine, mg/l, serum	156	54	25	14	15	16	27	54	86	120
Creatinine, mg/l, urine	900	400	850	2100	2200	1800	1700	400	530	580
Creatinine clearance, ml/min	22	47	64	78	64	35	7.3	1.6	1.4	3.6
Urine output, ml/24 h	3730	6700	2120	770	655	615	250	410	390	1130
Albumin in serum, g/l		36	—	—	31	30	28	—	27	24
Albumin in urine, mg/l	180	55	160	475	525	1225	4900	—	3710	1260
C _{alb} /C _{Cr} · 10 ³	0.58	0.15	0.10	0.09	0.11	0.43	4.0	—	27	11
α_2 -microglobulin, mg/l, serum	136	123		—	83	—	94	—	130	129
α_2 -microglobulin, mg/l, urine	140	82	160	228	170	39	37	—	94	88
C _{α} /C _{Cr} · 10 ²	12	7.1	3.9	1.8	1.3	0.46	0.86	—	14	15
β_2 -microglobulin, mg/l, serum	12.3	6.5		—	2.7	—	7.8	—	18.5	18
β_2 -microglobulin, mg/l, urine	95	33	75	119	21	8.3	6	—	56	56
C _{β} /C _{Cr} · 10 ²	90	53	37	24	3.7	1.5	1.4	—	58	70
Lysozyme, mg/l, serum	3.9	3.6	—	—	2.5	—	1.8	—	2.6	3
Lysozyme, mg/l, urine	5	1	2	2.3	1.4	0.7	1.2	—	2.8	2.2
C _{lys} /C _{Cr} · 10 ²	15	2.7	1.3	0.58	0.41	0.37	1.4	—	19.4	17
Total protein in urine, mg/24 h	3600	2680	800	1300	1350	2430	1850	—	1870	1510
Sodium, mEq/l, serum	140	140	140	149	142	145	140	140	138	140
Sodium, mEq/l, urine	56	33	72	39	16	14	46	74	60	68
FSE, %	4.7	2.3	1.1	0.18	0.08	0.12	0.78	9.3	8.4	10.7
Potassium, mEq/l, serum	4.6	4.6	4	4.9	4.6	4.3	5	6.1	5.7	4.3
Potassium, mEq/l, urine	51	13	49	80	65	90	72	33	40	22
FPE, %	129	30	29.3	11.3	10.4	24	35	98	155	107

ND: not detectable

GH*	EH*	AJ*	EK	KL*	HM*	EO*	MP	SP*	KP	IR	GS*	HS*	K
F	F	M	F	F	F	F	F	F	F	F	M	M	
51	20	73	47	41	70	52	52	47	48	61	60	64	
—	—	—	—	+	+	+	(+)	—	+	—	+	(+)	
no	no	no	no	yes	yes	yes	no	no	yes	no	yes	no	
yes	yes	yes	yes	no	yes	no	yes	yes	yes	yes	no	yes	
8	20	135	44	100	27	100	8	8	55	11	33	160	
88	37	4.1	15	4.7	20	3.5	100	102	7.3	80	22	1	
39	43	40	36	30	38	36	43	41	34	36	42	26	
20	152	350	720	2150	90	2270	24	30	885	11	23	740	
35	230	800	1000	2300	70	2200	25	50	1600	15	45	400	
0.008	0.12	3.4	1.5	12	0.075	15	0.004	0.009	4.2	0.003	0.03	13	0.0
67	138	171	128	158	98	129	53	39	166	58	131	—	
ND	2.1	73	54	108	7.5	59	ND	ND	19	ND	12	82	
—	0.05	16	3.3	11	0.2	7.5	—	—	1.8	—	0.46	—	
1.9	5.8	26	12	36	7.1	20	2.1	0.8	11	2	7.2	58	
ND	3.9	42	37	66	16	ND	ND	ND	13	ND	17	82	
—	2.3	62	25	30	8	—	—	—	19	—	12	64	
1.2	4.2	10	—	7.2	6.8	6	—	1.5	—	—	5.9	8.1	
ND	ND	1.4	—	3.6	1	2.1	—	ND	—	—	0.5	4.4	
—	—	5	—	8	0.45	7.5	—	—	—	—	0.4	25	

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
90	115	105	100	100	70	55	32	24	30	17	16	—	13	—	12	12	
850	630	510	450	380	425	450	450	500	480	450	300	300	430	480	400	380	4
0.3	3.4	6.3	10.4	8.3	8.6	11	22	29	37	46	55	48	45	56	57	65	
50	850	1845	3330	2665	1830	1370	1975	2275	2675	2505	3965	3200	1960	2190	2490	3100	27
—	25	—	27	—	26	—	—	18	—	29	—	31	30	—	—	30	
—	680	400	220	120	125	220	220	310	310	275	275	175	155	185	125	125	1
—	4.6	3.1	1.8	1	0.76	1	0.68	0.88	0.51	0.36	0.47	0.26	0.13	0.2	0.11	0.09	0.
—	116	—	100	—	120	—	—	115	—	111	—	104	100	—	—	103	
—	32	70	60	49	48	68	110	110	122	120	90	93	80	51	51	24	
—	4.7	15	13	9.5	6.0	5.7	5.9	5.2	5.4	4.2	4.2	4.3	2.4	1.4	1.6	0.75	0.
—	13.5	—	10.8	—	7.8	—	—	6.4	—	5.5	—	—	7	—	—	4	
—	2.2	16	16	16	18	21	20	24	33	43	33	30	32	25	16	15	
—	3.3	27	36	41	34	28	21	21	29	27	25	22	14	11	11	12	
—	2.1	—	1.8	—	1.3	—	—	1.7	—	1.1	—	0.9	0.8	—	—	1.05	
—	0.5	0.7	0.4	0.4	0.4	0.7	1.6	1.3	1.2	1.8	1.2	1.1	1	0.6	0.6	0.6	0
—	4.3	7.4	5.2	6.4	4.4	4.5	6	4.5	4.8	6.4	6.6	6.1	3.5	1.9	1.8	1.7	0.
—	600	350	195	170	ND	ND	ND	3400	5350	3250	6000	600	390	400	ND	ND	N
134	126	129	127	125	130	135	138	139	136	139	139	—	136	136	138	142	1
58	60	28	16	32	30	20	28	21	22	29	28	19	23	46	42	57	
5.4	8.3	4.4	2.8	5.6	3.3	1.4	1.3	0.82	0.8	0.79	1	0.65	0.51	0.91	0.9	1.3	0.
4.7	6.1	6.5	4.9	4.2	3.3	3.4	3.4	4.2	3.9	3.6	3.8	—	3.9	4.7	4.4	4	4
33	36	38	27	12	10	41	18	20	18	17	15	16	16	20	17	15	
74	99	133	133	73	45	118	29	27	24	17	20	19	11	12	12	12	

IS*	ES	IS	LV*	HÖ
F	M	F	F	F
28	51	25	60	62
—	(+)	—	(+)	+
no	yes	no	yes	no
yes	no	no	yes	yes
10	22	8	64	20
80	32	80	8	35
52	35	51	50	44
21	70	40	455	800
20	100	40	650	750
004	0.11	0.004	1.2	0.4
59	90	52	196	129
ND	9	ND	67	6
—	0.28	—	4.5	0.9
1.2	6.3	1.8	17	4.3
ND	27	ND	64	8.3
—	16	—	50	4.2
1.5	—	—	8	6.2
ND	—	—	7.7	1.7
—	—	—	13	0.6

29	30	31	32	33	34	35
13	—	12	12	—	12	—
80	350	450	330	430	425	350
72	66	83	55	76	79	96
00	3400	3200	2900	3050	3200	4750
—	23	—	24	—	—	30
48	86	126	109	151	145	57
11	0.09	0.10	0.12	0.12	0.12	0.06
—	110	—	111	—	—	112
26	22	21	23	30	44	18
61	0.71	0.5	0.76	0.76	1.11	0.57
—	3.8	—	3.9	—	—	4.1
16	14	14	16	20	21	11
11	13	9.7	15	14	15	9
—	1.2	—	1.2	—	—	1.2
13	0.2	0.4	0.6	0.8	0.7	2
57	0.59	0.88	1.8	1.9	1.6	5.7
D	ND	ND	ND	ND	ND	ND
42	143	144	141	—	141	—
52	66	65	73	40	88	90
99	1.7	1.2	1.9	0.8	1.8	2.2
1	4.4	4.7	4.7	—	4.6	—
22	22	20	15	17	20	50
14	17	11	12	10	12	37

